UNIVERSITI PUTRA MALAYSIA

ENZYMATIC INCORPORATION OF OLEIC ACID INTO REFINED BLEACHED AND DEODORISED PALM OLEIN

LIEW HAN-FANG

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By

LIEW HAN-FANG

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for papa with love

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman: Prof. Dr. Dzulkefly Kuang Abdullah, PhD

Faculty: Science

High oleic oils are very much in demand. Nutritionally, they are perceived to reduce cardiac related diseases. Oxidatively, they are more stable than polyunsaturated oils. However, palm olein, with natural oleic content of less than 50%, the olein solidifies in temperate climate. This study was conducted to investigate the performance of lipases on oil substrate to obtain high oleic content cooking olein.

In the first part of the work, the effect of several reaction conditions on lipase-aided acidolysis of palm olein with oleic acid was studied. Results showed no significant difference on the effect of molecular sieve added into acidolysis process. Studies on the other effects indicated that the optimal condition for *T. Lanoginosa* lipase was at 50 °C, 10 % (w/w) lipase loading and substrate concentration of 1:2 (POo:OA mole ratio). Eight hours was selected as the best reaction time. The acidolysis process also increased the slip melting point of palm olein after 8 h reaction with *T. Lanoginosa* lipase registering the largest increase (3.8 °C) compared to the initial unreacted

mixture. The catalytic stability of *T. Lanoginosa* lipase, after being subjected to ten runs of repeated usage indicated that the lipase can be reused to produce fairly constant products on a larger scale.

The rates of acidolysis were found to vary with different lipase sources. Generally both the *T. Lanoginosa* and *Alcaligenes sp.* lipases also produced the highest degree of acidolysis and % FFA, with oleic acid content up to about 60 %. Both lipase-catalysed mixtures cause the lift in slip melting point and solid fat content (SFC) at a higher temperature (above 25 °C) in all the two mixtures studied compared to the unreacted commercial olein.

The second part of this study, was studied the factors affecting the transesterification of palm olein with methyl oleate. Both lipases were used in acidolysis as a model to study the effect of temperature, lipase loading, substrate concentration and reaction time. The optimum condition for transesterification was at 50 °C, 10% (w/w) lipase load, substrate concentration of 1:2 (POo:MO mole ratio) and 2 h incubation time for both lipases. Similarly, the transesterification process also increased the SMP of palm olein after 2 h reaction with *T. Lanoginosa* lipase registering increase of 1.3 °C compared to the initial unreacted mixture. The SMP and SFC results share the similar trend with acidolysis, while the oleic acid content generated from transesterification was about 54 %, slightly lower than acidolysis.

Generally, the products obtained from interesterification contained higher oleic acid compared to that of starting POo (about 42-46%). Therefore, interesterification process without any further treatment, has improved the unsaturation of a palm oil product as well as monounsaturation content, which is comparable to olive oil.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGGABUNGAN ASID OLEIK SECARA ENZIM KEPADA MINYAK SAWIT DISULING DILUNTUR AND DINYAHBAU

Oleh

LIEW HAN-FANG

Jun 2007

Pengerusi: Prof. Dr. Dzulkefly Kuang Abdullah, PhD

Fakulti: Sains

Minyak tinggi oleik adalah sangat diperlukan. Dari segi zat makanan, ia mengerti kebolehan untuk mengurangkan kemungkinan sakit jantung. Dari segi pengoksidaan, ia adalah lebih stabil daripada minyak poli-tak-tepu. Bagaimanapun, minyak sawit olein mengandungi kandungan oleik kurang daripada 50% secara semulajadi, ini menyebabkan ia senang memepejal di negara-negara bermusim. Penyelidikan ini bertujuan untuk mengetahui perlaksanaan enzim dalam bahan minyak agar mendapat minyak masak berkandungan oleik yang tinggi.

Pada bahagian pertama bagi penyelidikan ini, kesan beberapa keadaan tindak balas asidolisis telah dikaji. Keputusan tidak menunjukkan perbezaan terhadap kesan saringan molekul yang telah ditambah. Pengajian terhadap kesan lain menunjukkan, keadaan optima bagi proses asidolisis oleh enzim *T. Lanoginosa* adalah, pada suhu 50 °C, sukatan enzim 10 % (jisim/jisim) dan kepekatan bahan tindak balas 1:2 (POo:OA nisbah mol). Masa tindak balas yang paling sesuai adalah 8 jam. Proses asidolisis menigkatkan takat lebur sorong (SMP) minyak sawit olein sebanyak 3.8 °C selepas tindak balas selama 8 jam dengan enzim *T. Lanoginosa*. Kajian atas

kestabilan mangkin bagi enzim *T. Lanoginosa* sebanyak 10 kali menunjukkan bahawa, enzim tersebut boleh dikitar semula dan masih mengekalkan penghasilan yang malar.

Kadar tindak balas asidolisis adalah berbeza antara sumber-sumber enzim yang berlainan. Lazimnya, kedua-dua enzim *T. Lanoginosa* dan *Alcaligenes sp.* dapat menghasilkan darjah asidolisis and % asid lemak bebas (FFA), dengan kandungan asid oleik sebanyak 60 %. Kedua-dua campuran hasil tindak balas mengakibatkan peningkatan takat lebut sorong (SMP) dan kandungan pepejal lemak (SFC) pada suhu yang melebihi 25 °C, berbanding dengan minyak sawit olein komersial.

Bahagian kedua bagi kajian ini adalah mengkaji faktor-faktor yang mempengaruhi tindak balas transesterifikasi minyak sawit olein dengan metil oleat. Kedua-dua enzim yang terlibat dalam asidolisis digunakan sebagai model untuk mengkaji kesan suhu, sukatan enzim, kepekatan bahan dan masa tindak balas. Hasil kajian menunjukkan keadaan optima bagi kedua-dua enzim adalah 50 °C, 10 % (jisim/jisim) sukatan enzim, kepetatan bahan 1:2 (POo:MO nisbah mol) dan masa tindak balas selama 2 jam. Begitu juga, proses transesterifikasi meningkatkan takat lebur sorong (SMP) minyak sawit olein selepas bertindak balas selama 2 jam degan enzim *T. Lanoginosa* sebanyak 1.3 °C. Keputusan takat lebur sorong (SMP) dan kandungan pepejal lemak (SFC) juga menunjukkan kecenderungan yang sama dengan asidolisis. Manakala, kandungan asid oleik yang terhasil dari transesterifikasi mencapai 54 %, agak lebih rendah daripada asidolisis.

Produk yang terdapat daripada interesterifikasi mengandungi asid oleik yang lebih tinggi daripada POo pada mulanya (42-46%). Maka, interesterifikasi dapat meningkatkan ketaktepuan minyak sawit dan khasnya kandungan mono-ketaktepuan.

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Last but not least, I would like to thank my family for their encouragement, understanding and patience in sharing my excitements and frustrations of research.

I certify that an Examination Committee has met on 8th June 2007 to conduct the final examination of Liew Han-Fang on her Master of Science thesis entitled "Enzymatic Incorporation of Oleic Acid into Refined Bleached and Deodorised Palm Olein" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Gwendoline Ee Cheng Lian, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Faujan Hj. Ahmad, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Md. Taufiq @ Yap Yun Hin, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Jumat Salimon, PhD
Associate Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(External Examiner)

HASANAH MOHD GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 16 August 2007

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

Dzulkefly Kuang Abdullah, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Siew Wai Lin, PhD
Senior Research Officer
Malaysian Palm Oil Board
(Member)

Cheah Kien Yoo, PhD
Senior Research Officer
Malaysian Palm Oil Board
(Member)

____

AINI IDERIS, PhD
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 13 September 2007

DECLARATION

I hereby declare that the thesis is based on my orinigal work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

LIEW HAN-FANG

Date: 15 August 2007

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LIST OF ABBREVIATIONS

12:0	lauric acid
14:0	myristic acid
16:0	palmitic acid
16:1	palmitoleic acid
18:0	stearic acid
18:1	oleic acid
18:2	linoleic acid
18:3	linolenic acid
AOCS	American Oil Chemist Society
area%	area percent
C46	mainly PMP
C48	mainly PPP
C50	mainly POP/PPO
C52	mainly POO and PLO
C54	mainly OOO
DAG	diacylglycerol
DSC	Differential scanning calorimetry
FA	Fatty acid
FAC	fatty acids content
FFA	free fatty acid
GC	gas chromatography
h	hour
HPLC	High Performance Liquid Chromatography

IV	iodine value
MAG	monoacylglycerol
ME	methyl ester
mins	minutes
MLP	1-myristoyl-2-linoleoyl-palmitoyl glycerol
MO	methyl oleate
mole %	mole percent
MPOB	Malaysian Palm Oil Board
MUFA	monounsaturated fatty acids
NMR	Nuclear Magnetic Resonance
OA	oleic acid
OLL	1-oleoyl-dilinoleoyl glycerol
OLO	1,3-dioleoyl-linoleoyl glycerol
OOO	tripalmitin
PLL	1-palmitoyl-dilinoleoyl glycerol
PLO	palmito-oleolinolein
PLP	1,3-dipalmitoyl-2-linoleoyl glycerol
PMP	myristodipalmitin
PO	Palm Oil
POO	dioleopalmitin
POo	palm olein
POP	dipalmitolein
POS	1-palmitoyl-2-oleoyl-stearoyl glycerol
PPP	tripalmitin

PPS	1,2-dipalmitoyl-stearoyl glycerol
PUFA	polyunsaturated fatty acids
RBD	refined bleached deodorized
SFC	solid fat content
SMP	slip melting point
SOO	1-stearoyl-dioleoyl glycerol
SOS	1,3 distearoyl-2-oleoyl glycerol
sp.	Species
SPD	Short Path Distillation
T. Lanoginosa	*Thermomyces Lanoginosa*
TAG	triacylglycerol
w/w	weight/weight

CHAPTER 1

INTRODUCTION

Palm oil is derived from the mesocarp of oil palm fruit, *Elaeis guineensis*, which originates from West Africa. In Malaysia, high yielding hybrid of Dura x Pisifera or Tenera is most commonly cultivated (Pantzaris, 1997). In the year of 2005, palm oil is the world's most widely produced (24% or 33.326 million tonnes) and consumed edible oil (21 million tonnes), slightly more than soybean oil (25.3% or 33.287 million tonnes), with Malaysia being the largest producer (45 %) and exporter (51.1 %) among palm oil output (MPOB, Oil World Annual, 1999-2005; Oil World Weekly, 16 Dec 2005; BCB, 2004). According to a forecast by Oil World (1994), the world production of palm oil projected to reach 25 million tones by the year 2008, but the numbers had exceeded, the projection showing the enormous potential in the global market. One of the challenges for the oil palm industry is to widen the application of palm oil.

Palm olein is the more liquid fraction obtained from palm oil fractionation after crystallization at a controlled temperature (Pantzaris, 1997). The co-product of the fractionation process is palm stearin, which is generally cheaper fraction used in shortening, margarine, and vanaspati manufacture (Pritchard 1969; Berger 1980; de Vries 1984; Idris *et al*. 1989), ice-cream manufacturing (Berger 1980), cocoa-butter substitute (Godin and Spensley, 1971). The physical characteristics of palm olein differ significantly from those of palm oil. Its fatty acids (FA) composition is 0.9-1.4% myristic acid, 37.9-41.7% palmitic acid, 4.0-4.8% stearic acid, 40.7-43.9% oleic acid, and 10.4-13.4% linoleic acid (Tan and Oh, 1981) while its TAG are mainly of C48 (1.3-4.0 mol%), C50 (37.7-45.4 mol%), C52 (43.3-51.3 mol%), C54 (7.0-12.6 mol%) and absence of C46 (Tan and Oh, 1981). With a narrow range of slip melting point (SMP) ranging between 19.0 and 23.0 ºC (Timms, 1985), palm olein is a natural liquid oil at ambient temperature, and is widely consumed as cooking and frying oil (Pritchard, 1969).

However, with its composition being prone to clouding, palm olein alone cannot withstand low temperature storage and exportation to temperate countries. Although technically there is nothing wrong with the oil quality, consumers tend to perceive cloudy oil as deteriorated product due to its unattractive appearance. Therefore, the retail market demands clear oils.

The fatty acid composition of edible oils (Hilditch, 1964) plays an important role in shelf life, nutrition and health. Oleic acid can be beneficial as monounsaturated fatty acid (MUFA) in reduction of low density lipoprotein (LDL) cholesterol without affecting the levels of high density lipoprotein (HDL) (Mattson and Grundy, 1985). Meanwhile, excessive saturated fatty acids diet, such as coconut oil (Ng *et al*, 1991), long chain saturated fatty acids (LSFA) diets (Kritchevsky *et al*., 1971), are observed to increase serum LDL cholesterol level, and contribute to atherosclerosis and carcinogenesis (Kubow, 1990), respectively.

There is considerable potential for direct competition in liquid oil market and higher nutritional value of palm olein if its physical and chemical properties can be modified. Processes such as double fractionation (Akaike, 1985), blending with